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LNP preparation

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An abbreviated version of this protocol was published in Science Advances in Nov 2020

CRISPR-Cas9 genome editing using targeted lipid nanoparticles for cancer therapy

DOI: 10.1126/sciadv.abc9450

Detailed protocol

LNP preparation:

1. Freshly dilute lipids stocks in absolute ethanol.
2. Mix lipid stocks in a glass vial using the following molar ratio: Ionizable lipid, DSPC, cholesterol, DMG-PEG, and DSPE-PEG at 50:10.5:38:1.4:0.1 in the desired total lipid concentration.
3. Load lipids to a 1mL Luer-lock syringe (BD Bioscience).
4. Dilute mCas9/sgRNA (mCas9:sgRNA 3:1 weight ratio, 1:10 molar ratio RNA to ionizable lipid) in a 25mM citrate buffer.
5. Load RNA solution to a 1mL Luer-lock syringe (BD Bioscience).
6. Load syringes into a NanoAssemblr microfluidic mixing device (Precision Nanosystems Inc.)
7. Mix solutions with the following conditions:
Solution volumes ratios- RNA solution: Lipids solution, 3:1.
Combined flow rate of 12 ml min^{-1}.
Waste flow through 0.1mL.
8. Load LNPs to a 14Kda dialysis tube (GeBa Bio-Applications).
9. Dialyzed LNPs twice for 16hr at 4°c against PBS (pH 7.4) to remove ethanol.
10. Collect LNPs and continue to following applications.

Materials:
DLin-MC3-DMA (MC3) and Lipid 8 were synthesized according to previously described methods (*1, 2*).
Cholesterol, DSPC (1,2-distearoyl-sn-glycero-3-phosphocholine), polyethylene glycol (PEG)–DMG (1,2-dimyristoyl-rac-glycerol), and DSPE (1,2-distearoyl-sn-glycero-3-phosphoethanolamine)–PEG were purchased from Avanti Polar Lipids Inc USA.
CleanCap Cas9 mRNA (modified) was purchased from Trilink Biotechnologies, USA.
sgRNAs were designed and synthesized by Integrated DNA Technologies Inc, USA.
PBS was purchased from Biological Industries, Israel.

References:

1. R. Kedmi, N. Veiga, S. Ramishetti, M. Goldsmith, D. Rosenblum, N. Dammes, I. Hazan-Halevy, L. Nahary, S. Leviatan-Ben-Arye, M. Harlev, M. Behlke, I. Benhar, J. Lieberman, D. Peer, A modular platform for targeted RNAi therapeutics. *Nature Nanotechnology* **13**, 214-219 (2018).
2. S. Ramishetti, I. Hazan-Halevy, R. Palakuri, S. Chatterjee, S. Naidu Gonna, N. Dammes, I. Freilich, L. Kolik Shmuel, D. Danino, D. Peer, A Combinatorial Library of Lipid Nanoparticles for RNA Delivery to Leukocytes. *Advanced Materials* **32**, 1906128 (2020).

How to cite:（Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used）

1. Gutkin, A. and Rosenblum, D. (2020). LNP preparation. Bio-protocol Preprint. bio-protocol.org/prep690.
2. Rosenblum, D., Gutkin, A., Kedmi, R., Ramishetti, S., Veiga, N., Jacobi, A. M., Schubert, M. S., Friedmann-Morvinski, D., Cohen, Z. R., Behlke, M. A., Lieberman, J. and Peer, D.(2020). CRISPR-Cas9 genome editing using targeted lipid nanoparticles for cancer therapy. Science Advances 6(47). DOI: 10.1126/sciadv.abc9450

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